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# The first reported occurrence of Mycoplasma conjunctivae in goats in the Ningxia Hui Autonomous Region, China: A case report

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**Case Report**

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## Abstract

# Background

*Mycoplasma conjunctivae* (*M. conjunctivae*) belongs to the Mycoplasma species of the Mycoplasma family and it is the main pathogen that causes infectious keratoconjunctivitis in goats. Infectious keratoconjunctivitis (IKC), also known as red eye disease, is a highly contagious disease that is prevalent worldwide.

# Case presentation:

In October 2022, a goat farm in Tongxin County, Ningxia Hui Autonomous Region, China began to show leukoplakia in the eyes of goats 3 days later due to the introduction of a batch of goats and no isolation observation. The diseased goat showed listlessness and poor appetite. Its body temperature was as high as 41°C. The eyes began to appear white spots, and its range gradually expanded, covering the entire eyeball. 200 goats were purchased, 40 goats were affected, the incidence rate was 20%. Eye secretions of 15 diseased goats were collected and were amplified by using specific primers. The amplified products were sequenced and phylogenetic trees were constructed. The phylogenetic tree showed that Mco 01, Mco 02, Mco 03, Mco 04, Mco 05, Mco 06, Mco 07, Mco 08, Mco 09, Mco 10, Mco 11, Mco 12, Mco 13, Mco 14 and Mco 15 had the closest genetic distance with the reported *M.conjunctivae* Goat 655 and M165 / 69 strains, and the self-test support rate with Goat 655 was as high as 98.

# Conclusions

This study is the first to identify *M.conjunctivae* from the eye secretions of goats with IKC in goats from Ningxia Hui Autonomous Region. Under the stress conditions of long-distance transportation, the health status of goats should be highly concerned, and timely prevention of *M. conjunctivae* disease can improve the survival rate of purchased goats.


## Background

*Mycoplasma* is intermediate in size between viruses and bacteria and presents a high degree of pleomorphism, with the vast majority being round [1]. Because it is the smallest prokaryote lacking a cell wall, it is insensitive to antibiotics (such as penicillins and cephalosporins) acting on the cell wall [2]. *Mycoplasma* has contact and infectivity, and its host range is wide, which can cause *Mycoplasma* synoviae disease, *Mycoplasma* gallisepticum disease, *Mycoplasma bovis* disease, *Mycoplasma ovipneumoniae* and infectious keratoconjunctivitis (IKC) [3].

*Mycoplasma conjunctivae* (*M. conjunctivae*) belongs to the *Mycoplasma* species of the *Mycoplasma* family and it is the main pathogen that causes IKC in goats [4, 5]. *M.conjunctivae* was first isolated from

the eyes of diseased goats in Australia by Surman in 1968. Four years later, Barile M F isolated the isolation from the eyes of diseased goats in Maryland and named it as *M.conjunctivae* [6] IKC can be transmitted through direct contact with eye and nasal secretions of infected animals, or indirect contact with mosquitoes, flies, contaminated utensils and feed [7]. In the early stages of the disease, the unilateral eye of the sick animal is infected, and the affected eye is shy and tearing, the eyelids are semi-closed, and the eye droppings are increased accompanied by purulent secretions [8]. With the continuous development of the disease, to the late stage, the disease will affect the other side of the eye, resulting in binocular infection. At this time, the cornea was red, swollen, congested, raised and turbid, gradually thickened and milky white and the protrusions covered the entire eyeball in a cloud shape [9]. Due to the aggravation of the disease, the cloud-like substance fell off, ulcerated and corneal perforation occurred, resulting in irreversible damage and permanent blindness [10]. IKC disease, also known as red eye disease, is a highly contagious disease that is prevalent worldwide. It has been reported in Switzerland [11], Finland [12] and Spain [13]. Different breeds of goats can be infected with the disease [7]. Once animals are infected with this disease, the light ones have difficulty in feeding and inconvenient movement, resulting in fat loss, increasing weight loss, and low feed conversion rate, which seriously damage the interests of farmers; the heavy ones are in worse condition because they cannot find food, cannot maintain the wild survival, and even fall off the cliff [14].

## Case presentation

In October 2022, a goat farm in Tongxin County, Ningxia Hui Autonomous Region, China began to show leukoplakia in the eyes of goats 3 days later due to the introduction of a batch of goats and no isolation observation. The diseased goat showed listlessness and poor appetite. Its body temperature was as high as 41°C. The eyes began to appear white spots, and its range gradually expanded, covering the entire eyeball. 200 goats were purchased, 40 goats were affected, and the incidence rate was 20% (Fig. 1). Through the diagnosis of clinical symptoms and autopsy results, it is preliminarily suspected that it is Infectious keratoconjunctivitis caused by *Mycoplasma conjunctivae*. To determine the cause of this disease, eye secretions of 15 diseased goats were collected.

Two mL eye swab suspension was taken and centrifuged at 12 000 r/min for 2 min, and genomic DNA was extracted using a bacterial genomic DNA extraction kit (Tiangen, Beijing, China). The specific primers (McoF1: 5’-GTATCTTTAGAGTCCTCGTCTT TCAC-3’; 5’-CAGCGTGCAGGATGAAATCCCT C -3’) were designed according to Giacometti M [16]and synthesized by Bioengineering (Shanghai) Shanghai Co., Ltd.

The PCR reaction system was a 50 μL system, 2 μL template, 1 μL upstream and downstream primers, 25 μL 2 × Tap Master Mix, 21 μL dd$H_2O$. Reaction conditions: 94°C 2 min; 94°C 30 s, annealing 30 s, 72°C 1 min, 35 cycles; extension at 72°C for 8 min. The PCR amplification products were analyzed by 1% agarose gel electrophoresis, and the PCR products were purified by gel recovery kit and were sent to Bioengineering (Shanghai) Shanghai Co., Ltd. for sequencing. The sequencing results were spliced by DNA star software, and the spliced sequences were compared by the BLAST program on NCBI. The

phylogenetic tree was constructed based on the spliced sequences by using the Neighbor-joining (NJ) method in MEGA7.0 software. The phylogenetic tree showed that *Mco 01, Mco 02, Mco 03, Mco 04, Mco 05, Mco 06, Mco 07, Mco 08, Mco 09, Mco 10, Mco 11, Mco 12, Mco 13, Mco 14* and *Mco 15* had the closest genetic distance with the reported *M.conjunctivae Goat 655* and *M165 / 69* strains, and the self-test support rate with *Goat 655* was as high as 98 ( Fig. 2 ). It has a distant relationship with *Mycoplasma hyorhinis*, *Mycoplasma dispar* and *Mycoplasma ovipneumoniae*. As a result, these isolates were further verified to belong to *M. conjunctivae.*

# Discussion and conclusions

In this study, we first detected *M.conjunctivae* in goats from Ningxia Hui Autonomous Region. The diseased goats showed clinical symptoms such as poor appetite, frequent blinking, corneal opacity, ulcers and even blindness, which was highly consistent with the reports on the clinical symptoms of wild goats and antelope written by Marco I [15]. The symptoms such as frequent tears, increased eye feces, mild conjunctivitis, or corneal opacity in both eyes were observed in diseased goat, which was very similar to the typical clinical symptoms of small ruminants reported by Fernandez-Aguilar X [16]. After observing the diseased goat, it was found that the clinical symptoms were mildly manifested as continuous tears, conjunctival congestion and peripheral corneal edema; in severe cases, it is manifested as keratitis, corneal ulcer and even corneal perforation, resulting in permanent blindness. The above is the same as the report of infectious corneal conjunctivitis in goat was reported by Williams H J [17]. This study revealed that the clinical symptoms of captive goats infected with *M.conjunctivae* were the same as those of small ruminants such as wild goats, antelope and goats.

*Mycoplasma* can escape the host's immune response in various ways, thus surviving in the host body [18]. Almost all of these escape processes can cause changes in the structure and expression function of *mycoplasma* surface membrane proteins, so these mechanisms are essential for *mycoplasma* to adapt to the host and chronic colonization in the host [19, 20]. Once the *mycoplasma* is colonized in the body, it is difficult to completely remove it, which makes the body vulnerable to the invasion of other pathogens and easy to mix with other pathogenic microorganisms. For example, cases of mixed infection with pathogens such as *Rickettsia* [21], *Mycoplasma agalactiae* [22] and *Chlamydia psittaci* [23] have been reported. Therefore, under the stress conditions of long-distance transportation, feed replacement and environmental change, we should attach great importance to the health status of goats and do a good job in the prevention of various diseases in time to improve the survival rate of purchased goats.

In China, long-distance transportation between livestock is an important factor in the spread of animal diseases [24]. On the one hand, due to the stress response of long-distance transportation and the inadaptability of climatic conditions, the resistance of the animal body is reduced, so some conditional pathogenic bacteria breed in large quantities, which leads to the emergence of animal epidemics. On the other hand, the detection was not strictly strengthened before the introduction, and isolation observation was not implemented after the introduction. Due to the introduction back is captive breeding, breeding density is larger, poor ventilation, and poor management, bacteria, and viruses will take the opportunity to

enter and cause disease. In addition, the genetic differences between animal individuals may also affect the occurrence, development and prognosis of the disease [25]. This batch of goats has introduced back to Ningxia autonomous region from another province, and the related diseases were not detected at the time of introduction, and the isolation observation was not paid attention to after introduction. Therefore, to avoid similar situations, disease detection should be strengthened before introduction, and isolation should be carried out under the relevant requirements of *veterinary laws and regulations* after introduction. The quarantine period for large and medium-sized animals is 45 days, and the quarantine period for small animals is 30 days. During the quarantine period, a suitable environment such as dry, clean and well-ventilated should be provided, and anti-stress drugs should be given to animals to minimize the possibility of epidemic disease.

# Abbreviations

IKC
Infectious keratoconjunctivitis
DNA
Deoxyribonucleic acid
PCR
Polymerase chain reaction
NCBI
National center for biotechnology information
NJ
Neighbor-joining

# Declarations


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### Authors' contributions

YNG and HYL performed the experiments and analyzed the data and drafted the manuscript. JSW revised the manuscript. JDW designed the experiments. All authors read and approved the final manuscript.

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### Availability of data and materials

The datasets generated and/or analysed during the current study are available in the National Center for Biotechnology Information repository (OQ983580- OQ983594)

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Written informed consent for publication of the clinical details and images of this case was obtained from the owners.

### Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and /or publication of this article.

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# Figures

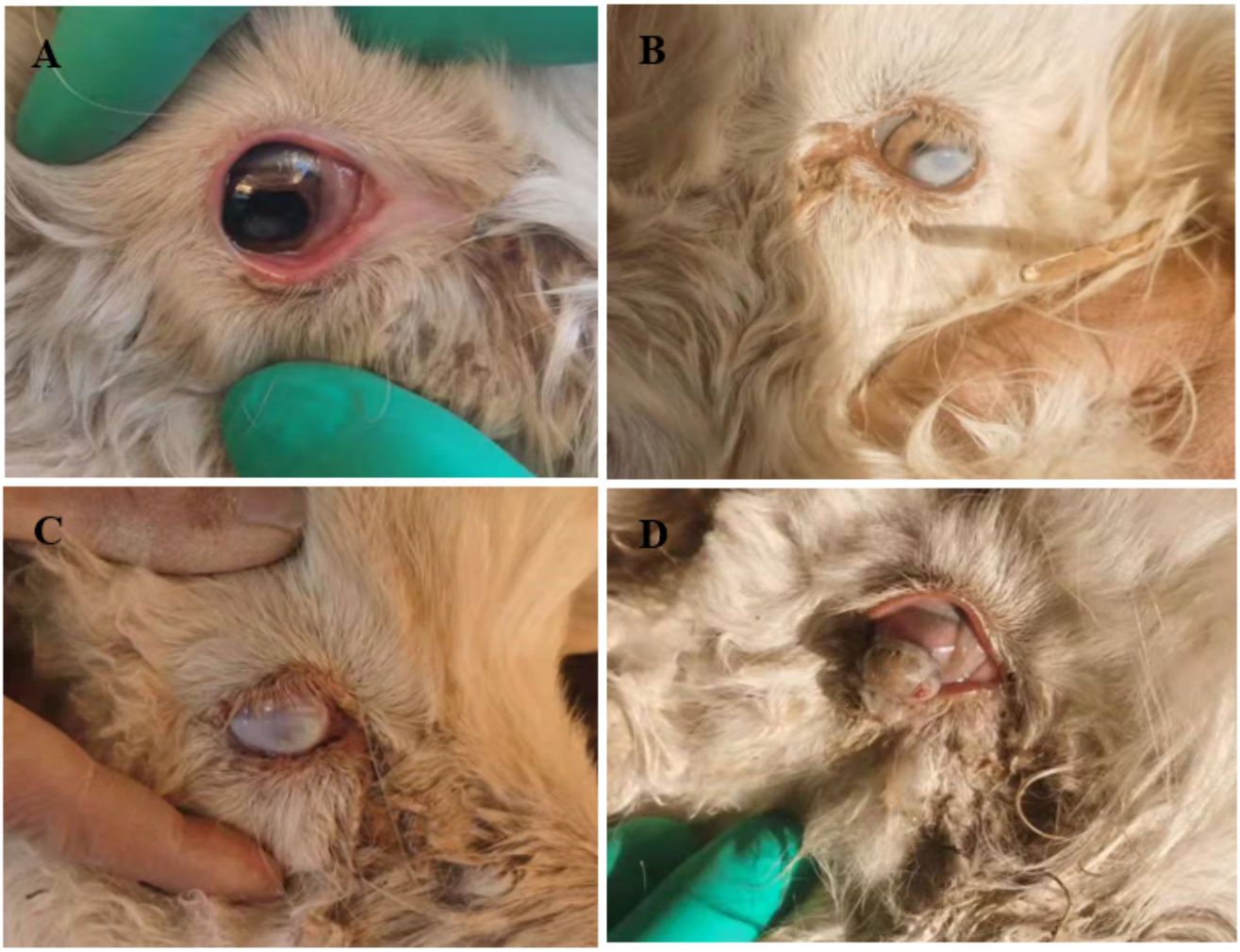
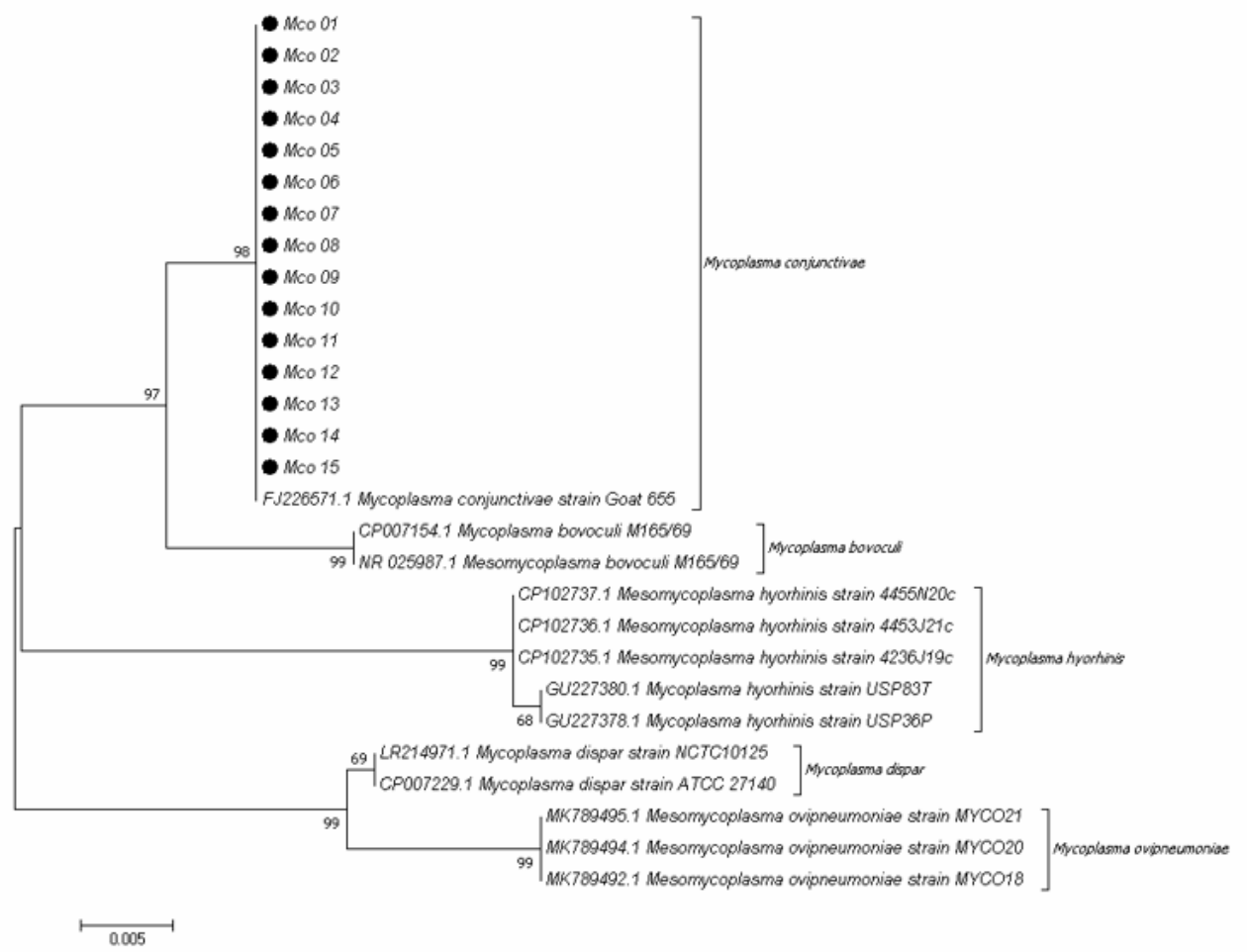



**Figure 1**

Pathological changes in the eyes of diseased sheep




## Figure 2

Phylogenetic tree constructed from *Mco* specific primer gene sequences